Total Orthotopic Small Bowel Transplantation In Swine Under FK 506

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PREVIOUS experimental studies in rodents and in dogs have established the efficacy of FK 506 in controlling the immunologic events following small bowel or multivisceral transplantation.[1-5] To complete the assessment of FK 506 in experimental small bowel transplantation, we present here our experience with the frequently used swine model.

MATERIALS AND METHODS

Fifty-two outbred female piglets (mean weight 29.3 ± 2.1 hg) were used, half as donors and half as recipients. Unlike the dog model, total orthotopic small bowel transplantation in the swine is not possible with the exchange of grafts between the donor and the recipient because of vascular anatomy.

Under general anesthesia with isofluorane, the entire donor's small bowel except the duodenum and 5 cm of the terminal ileum was isolated with its vascular pedicle and excised. The graft was perfused through the aorta with cold University of Wisconsin solution. The transplantation technique was essentially the same as that originally described by Kumlin et al.[6] The superior mesenteric artery and the superior mesenteric vein were anastomosed end-to-end. The proximal intestinal continuity was restored with a side-to-side anastomosis between the recipient duodenum and the end of the first jejunal loop of the graft. A 10-cm segment of the graft-proximal jejunum was exteriorized, as a stoma, for visual monitoring and postoperative mucosal biopsies. The distal intestinal continuity was obtained with an end-to-end or a side-to-side anastomosis between the extremity of the graft ileum and the last 5 cm of recipient ileum. A gastrostomy was performed to allow enteral feeding and oral FK 506 administration.

The animals were divided into five groups, according to treatment (Table 1). Five animals (one of each group) who died within 8 days from various technical causes were excluded from the final analysis. Treatment was maintained until death occurred. The animals who became lethargic or lost more than 40% of their preoperative body weight were killed with an anesthesia injection. FK 506 trough levels were determined from whole blood by a fluorescence polarization immunoassay (FPIA, TDX-Abbott). Antibiotic prophylaxis consisted of ceftazidime 3 g/d.

Postoperative monitoring was based on observations of the clinical course, stools, appearance of the stoma, body weight, blood chemistries, body temperature, blood cultures, and mucosal biopsies. Tissues obtained from stomal biopsies or at postmortem examination were fixed with 10% buffered formalin and stained with hematoxylin-eosin. Histologic features of acute rejection are reported in detail in another part of this issue.[7] Maltose absorption test (1 g/kg) was performed periodically using the modified method of Billiar et al.[8]

Table 1.

Group	No.	FK 506 mg/kg d	FK 506 Route	Steroids	Mean survival (d)	Best survival (d)
1	3	—	—	—	12 ± 1	13
2	5	0.1	IM	Yes*	12 ± 3	17
3	5	0.15	IM	Yes*	18 ± 1	19
4	8	0.2–0.3	IM†	No‡	27 ± 6	35
5	5	0.3	IM + OS§	No‡	32 ± 12	45

*Bolus at reperfusion (500 mg IV), recycle, maintenance dose (10 mg kg/d IM).
†After 3 to 7 d of 0.2 mg·kg d, the dose was increased to 0.3 mg·kg d.
‡Bolus at reperfusion (500 mg IV).
§FK 506 was administered IM for the first 2 postoperative weeks, then orally at the same dose.

RESULTS

The best mean survival rate occurred in group 5 (32 ± 12 days) with two animals that survived more than 6 weeks. One animal is still alive in good condition at 23 days. Causes of death were emaciation (two cases) and strangulated obstruction (one case). In group 4 the mean survival was 27 ± 6.5 days. Lethal emaciation and sepsis caused the death of all animals but one, which died from acute rejection.

All untreated recipients (group 1) died of acute rejection within 12 days (mean 12 ± 1 days) as well as animals of group 2 (mean 12 ± 3 days). In group 3 survival was prolonged to a mean of 18 ± 1 days. All recipients of this latter group experienced acute rejection, which caused the death in 50% of the cases whereas in the other 50% it contributed to the development of fatal sepsis. The five animals (one for each group) excluded from the final analysis died between days 3 and 8 from technical complications such as volvulus (two cases), arterial thrombosis (two cases), and intraperitoneal hemorrhage (one case). Clinical or histologic graft-vs-host disease (GVHD) was not seen in any of the animals.

A detailed evaluation of the incidence and the severity of the rejection episodes in groups 1 to 4 is reported in another part of this issue.[7] The results of maltose absorption test are also explained elsewhere in this issue.[9]

FK 506 trough levels for each group are shown in Fig 1. They were at a very low range in groups 2 and 3 with a

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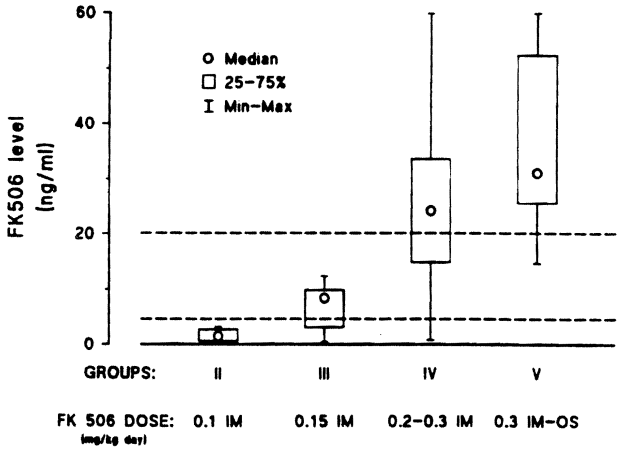

Fig 1. FK 506 levels in each study group.

mean of 1.7 ± 1.1 ng/mL and 6.8 ± 4.2 ng/mL, respectively. In groups 4 and 5, FK 506 levels were increased to a mean of 26.1 ± 13.4 ng/mL and 36.2 ± 15.3 ng/mL, respectively. Interestingly, in group 4 the peak level was reached between the first and the second week in accordance with the increased intramuscular (IM) dose (see Table 1). In group 5 the peak level was obtained during the first week and, after the conversion to the oral route, it decreased and was maintained stable to a mean of 24.1 ± 6.2 ng/mL.

DISCUSSION

Previous experimental studies have assessed the feasibility of small bowel transplantation in pigs.[10] Long-term survivals have been achieved with cyclosporine (CyA)-based immunosuppression.[11,12] However, acute rejection was controlled with such high doses of CyA, corresponding to almost five times those tolerated by humans.[11,12]

FK 506 has already been tested in a large animal model, the dog.[5] This is the first study on pigs to assess the efficacy of FK 506 for small bowel transplantation. Four different dosages of FK 506 were tested. The choice of the IM route was made to simplify the postoperative management of the animals. Pharmacokinetic studies demonstrated that after IM injection the drug is well absorbed and its 12-hour kinetic profile curve is almost equivalent to a continuous IV administration of a lower dose (unpublished data).

Doses of FK 506 of 0.1 or 0.15 mg/kg/d IM were not sufficient to prevent acute rejection, even if steroids were added. When FK 506 was given at doses of 0.2 mg/kg/d, rejection still occurred within the first 2 weeks. For this reason, the immunosuppressive protocol was modified in group 4 animals and, after a 3-day course of IM FK 506 at 0.2 mg/kg/d, the dose was increased to 0.3 mg/kg/d. Survival improved and most of the animals reached a month. However emaciation associated in most cases with sepsis became the common cause of death in this group. Only one recipient developed acute rejection. At the postmortem examination, all other animals were rejection free but five of seven presented scattered polypoid lesions in the graft, a possible sign of lymphoproliferative hyperplasia.[7] No other similar lesions were found in other organs.

After a loading dose of 0.3 mg/kg/d, conversion to the oral route was made after 7 to 10 days in group 5 animals. Of the five recipients, two died for technical complications whereas two others registered the best survival in the present study: 42 and 45 days, respectively. The cause of death of these last two animals is still controversial because the postmortem histologic evaluation is not yet available. They maintained normal weight and good absorptive function (confirmed by the FK 506 trough levels) until 5 to 7 days before death and then suddenly worsened and died. Both animals had positive blood cultures for Streptococcus at the 30th postoperative day. Macroscopic signs of severe acute rejection were absent in the graft at the postmortem evaluation. However the intestinal wall of both grafts appeared thicker when compared with that of the recipient intestine. Moreover, FK 506 levels dropped significantly after conversion to oral route and this fact may have predisposed to chronic rejection. A higher FK 506 oral dose could be necessary to prolong survival in this group. The surviving animal is now being treated with oral 0.4 mg/kg/d.

Sepsis has been a major problem in the management of all animals and it affected survivals significantly. Many recipients already had positive blood cultures before surgery. Our opinion based on this early experience is that swine is a difficult species for studies in organ transplantation. However, FK 506 can provide powerful immunosuppression after small bowel transplantation at the suggested doses (group 4 and 5), allowing a good survival of the animals for more than a month. As a consequence, this model can be used for morphologic, functional, and immunologic studies.[7,13,14] We think that longer survivals can be achieved with better monitoring of FK 506 levels and better control of the infectious complications.

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